Intra-Uterine Healing of Fetal Rat Cheek Wounds

BRUCE W. ROBINSON B.D.S., B.Sc. Dent. (Hons).
ALASTAIR N. GOSS D.D.S., FRACDS.
Adelaide, South Australia 5000

Cheek wounds, involving skin, mucosa, and muscle, were surgically created in utero on Sprague Dawley rat fetuses at 19½ days gestation. Fifty fetuses were used in the study. Twenty-five fetuses were in the experimental group and 25 in the control. Five fetuses in each group were sacrificed at 0, 6, 12, 18 and 24 hours post-operatively. Serial histological sections in the coronal plane were stained with haematoxylin and eosin.

Restoration of continuity and primary epithelialisation of the oral mucosal and skin surfaces of the wound was observed to occur within 24 hours. The continuity of the muscle layer was not restored, but some mitoses were seen in the myeloblasts. There was a complete absence of acute inflammatory cells, and there was no scab formation. Healing occurred without scarring. These findings are discussed in comparison to previous findings on orofacial fetal wound healing (Goss 1976) and in regard to future prospects in intraceptive fetal surgery for the correction of deformities, in particular *cleft lip.*

KEY WORDS: Cheek wounds, fetuses-rat, healing, cleft lip.

Introduction

Surgical correction of cleft lip always results in greater or lesser degree of residual scarring and deformity. Recent studies have indicated that these residual sequelae may be much less if healing occurs *in utero* (Sopher 1975).

Fetal wounds heal in a broadly similar way to postnatal wounds, but there are important differences (Sopher 1975, Goss 1977). The progression of epithelial division, migration, and differentiation, (Viziam 1964) is essentially the same pre- and post-natally. However, fetally, the rate is measured in hours rather than days as is the case for adult wounds. Although this trend is present in all the reports on fetal wound healing, there is a range in the rate of the epithelial response (Hess, 1954; Somasumdaram and Prathap, 1970, 1972; Sopher, 1975; and Goss, 1977). These differences may be related to the fact that different species, ages, sites, and wound types were involved.

The most striking difference, however, between pre- and post-natal wounds is the complete lack of a cellular infiltrate in the fetal wounds. In adult wounds, a cellular inflammatory response is always present, and many believe that it is essential for normal healing (Viziam, 1964; Ross, 1969).

In the only other study of orofacial fetal wound healing in rodents (Goss 1977), a complex wound involving both hard and soft tissues in the cranium, nasal chamber, and mouth was studied. In this experiment, a simpler wound involving only orofacial soft tissues was studied.

Materials and Methods

Mature Sprague-Dawley rats were kept under conditions of controlled light and temperature, on a stock diet of rat pelletts and water "*ad libatum.*" Animals were mated overnight and day zero of gestation taken as midnight on the day of separation.

Ten pregnant rats were used. At 19½ days gestation, the rats were anaesthetised with intraperitoneal chloral hydrate 400 mg/Kg body weight (Lehmann et al 1938).

A laparotomy was performed under aseptic conditions, and both gravid uterine horns were lifted out of the abdominal cavity and placed on sterile drapes. A microdissecting opthalmic knife was then carefully passed through the uterine wall and amniotic membrane to enter the fetal mouth anterior to the right oral commissure. The knife was carefully advanced posteriorly through the buccal tissue to a point anterior to the facial artery; thus creating an incised wound of the full

Mr. Robinson is a postgraduate student and Dr. Goss is Chairman and Senior Lecturer, Department of Oral Pathology and Oral Surgery, University of Adelaide, North Terrace, Adelaide, South Australia 5000.

thickness of the cheek (Figures 1, 2 & 3). Alternative fetuses in both uterine horns were operated upon, thus leaving the remainder of the fetuses to be used as controls. The uterine horns were then replaced in the abdominal cavity and the laparotomy wound closed in layers.

Fifty fetuses were used and were divided into groups of five experimental and five control animals for each of five time periods (0, 6, 12, 18 and 24 hours post operation). All fetuses were examined for viability, malformations, and the effect of surgery on the face. They were fixed in 10% formol-saline for 24 hours and the face and cranium processed for histologic examination. Serial sections were cut in the coronal plane 7μ thick and were stained with haemotoxylin and eosin.

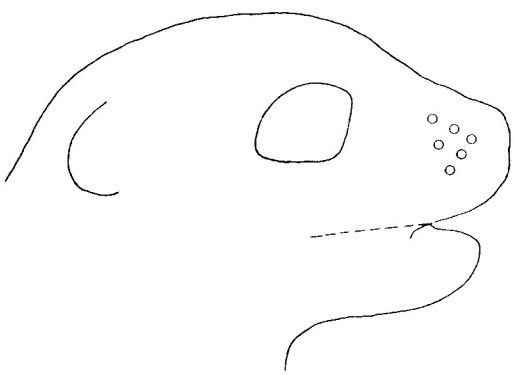

FIGURE 1. Lateral aspect of a rat fetus, 19½ days in utero, showing extent and position of incision (- - - - - -).

Results

Essentially similar changes were observed in each group, so a composite gross and microscopic description for each group is given.

Grossly, at zero hours, the orofacial wound was clearly evident. It extended from the oral commissure to the masseter region. The wound gaped open so that the tongue and alveolus could be seen. Observations made at six-hour intervals showed rapid closure. After 24 hours, the orofacial incision had disappeared without apparent scarring (Figure 4). At no stage was a scab observed.

The gross observations were supported microscopically. At zero hours, the path of the knife could be traced through the cheek tissue from the oral commissure to the mandibular ramus. In all cases, the knife created a full-thickness would involving mucosa, muscle, and skin. The oral and skin edges were lifted from the underlying mesenchyme. This adjacent mesenchymal tissue was hyperaemic, and the wound breach itself was filled with haemorrhagic debris (Figure 3).

At six-hour intervals thereafter, epithelial mitosis, followed by migration and differen-

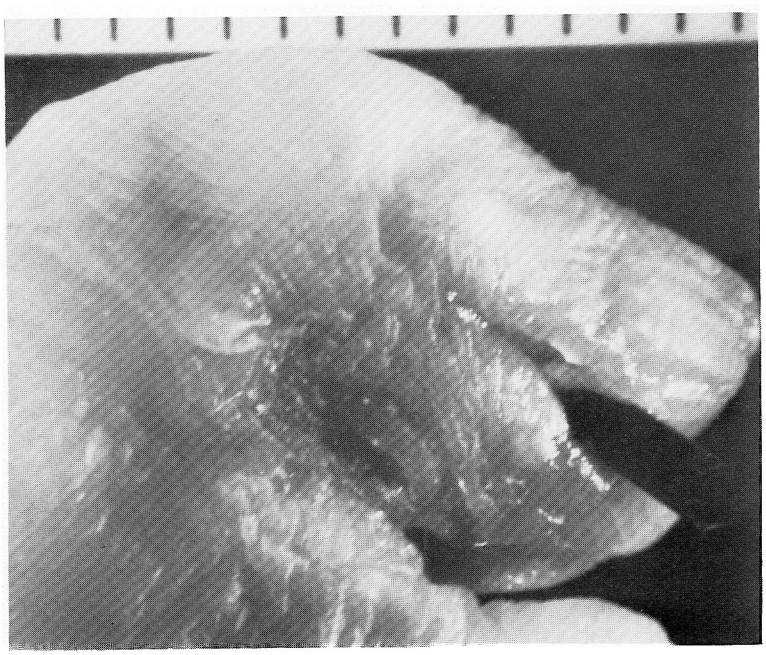

FIGURE 2. Lateral view of rat fetus 19½ days in utero, showing microdissection knife and extent of orofacial wound at zero hours. Scale in mm. Constant magnification.

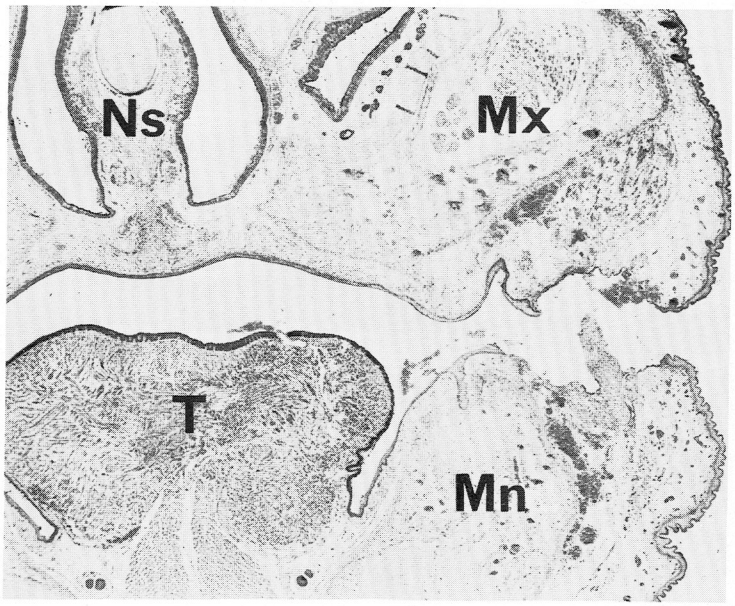

FIGURE 3. *Photomicrograph* illustrating cheek wound zero hours post-operatively. Coronal plane showing relationship of wound to mandible (Mn), maxilla (Mx), tongue (T) and nasal septum (Ns). (H & E × 25.)

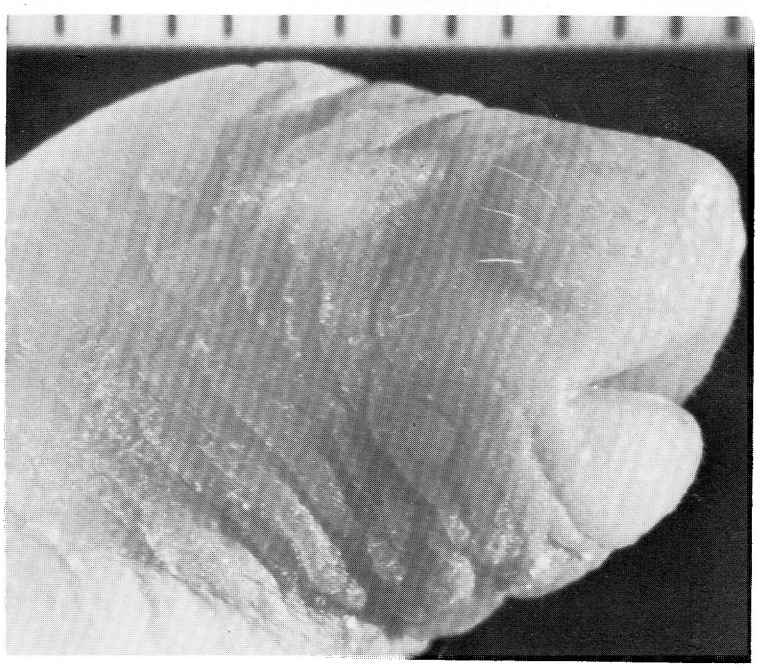

FIGURE 4. Lateral aspect of rat fetus 20½ days in utero, showing a completely healed 24-hour, post-operative orofacial wound. Scale in mm. Constant magnification.

tiation, was observed on both epidermal and mucosal epithelial surfaces. After 24 hours, epithelial continuity had occurred on both surfaces (Figure 5). The epidermis had in most cases resumed its normal state and was eight to 10 cells thick with surface keratinisation and the presence of rete pegs. The oral mucosal epithelium, though primarily closed, varied in thickness from one to five cells. In some specimens, basal triangular proliferations of dividing cells were seen (Figure 6).

The mesenchymal connective tissue components of the cheek showed progressive healing. However, at 24 hours, partial disruption

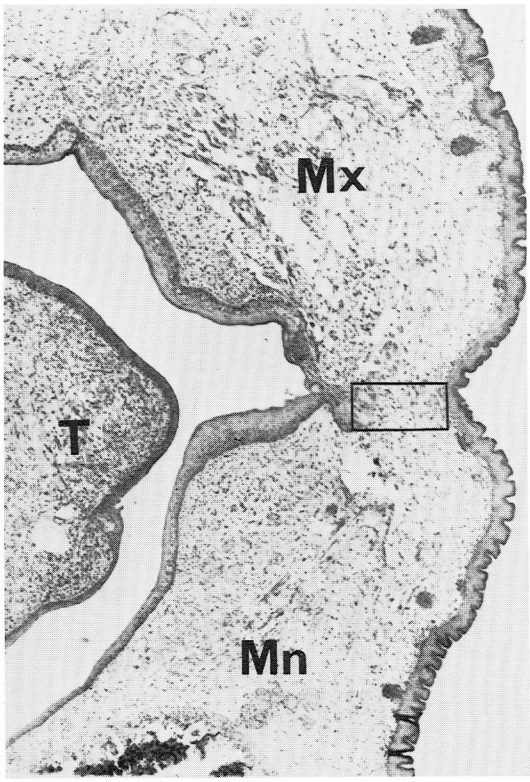

FIGURE 5. Photomicrograph of 24-hour post-operative cheek wound. Epithelial continuity complete on both skin and mucosal surfaces. Buccinator muscle retracted and inflammatory oedema present in mesenchymal wound area. (H & E × 40.)

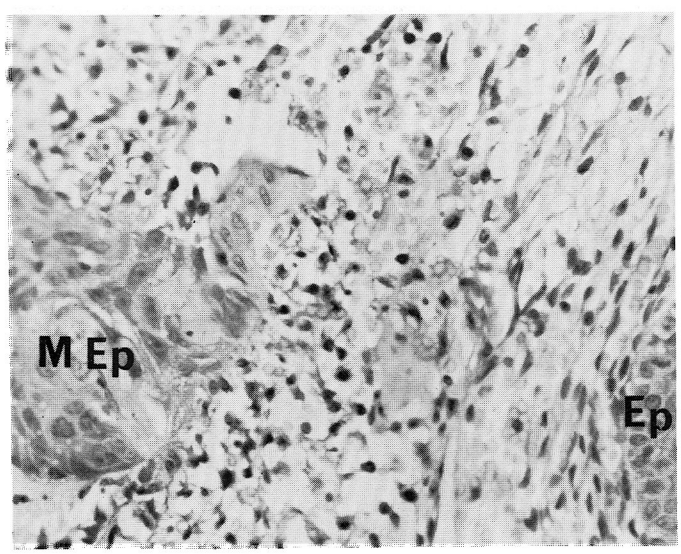

FIGURE 6. High-power photomicrograph of wound area outlined in Figure 5. Differentiation of skin epithelium complete but mitosis and differentiation still in progress in the mucosal epithelium. No recognisable acute inflammatory cells present in the wound area. Epidermis (Ep), mucosal epithelium (M.Ep.). (H & E × 400.)

and evidence of inflammatory edema was still apparent (Figure 5). No recognisable acute inflammatory cells were present at any stage. The divided muscle had retracted away from the incision, and no return to continuity was seen. After 12 hours, myeloblasts undergoing mitosis were recorded in some sections in the muscular elements of the cheek.

Discussion

These results show that rapid primary closure of a relatively large incised cheek wound

occurs within 24 hours. This finding is consistent with the general pattern of fetal wound healing (Hess, 1954; Dixon, 1960; Somasundaram and Prathap, 1970, 1972; Sopher, 1975; and Goss, 1977). Common features of particular note are the rapid progression of epithelial changes; the absence of scab formation and scarring; and a cellular inflammatory reaction. The absence of these normal components of postnatal healing can be ascribed either to the immaturity of the tissues, in particular of the reticuloendothelial system, or to the sterile environment rendering a defense mechanism unnecessary. In the near-term fetal rat, we believe that this latter suggestion is most likely. The protective environment and thus the lack of irritation or infection, would account for the lack of scarring. An important role of the scab in postnatal wounds is to prevent continued loss of body fluids through the breach epithelium. As fetal wounds are in a sterile environment, there is no need for a scab.

A feature of note of this study is that the rate of surface closure was even faster than that previously described for fetal wounds. All of the other studies, with the exception of that by Goss (1977), involved trunk skin wounds so that regional anatomical differences may be involved. Certainly adult skin wounds in the face heal faster than those on the trunk or limbs. These differences are usually attributed primarily to the differences in blood supply, which would also be important fetally.

In Goss's study (1977), the initial wound was more complex as it involved both hard and soft tissue of the cranium, nasal passages, and hard palate. The exposed surface of palatal mesenchyme took up to 72 hours before epithelial coverage was complete. This apparent difference in rate might be related to the fact that, in the present study, the edges of the incised wound were spontaneously opposed. In the earlier study, craniofacial growth tended to distract the edges of the palatal shelves, thus increasing the size of the wound. The difference in site may also be important as the rate of epithelialisation is faster in the buccal mucosa than in the palate in adult rats (Toto et al., 1966; Alvares et al., 1971).

The healing of fetal muscle wounds has not previously been reported. Although the muscle ends did not show any sign of reconstituting in this study, it is noteworthy that some mitotic activity was observed in the myeloblasts. Thus, at this stage, healing by regeneration rather than by scarring may be possible. Further long-term studies would be necessary to confirm this hypothesis.

The rapid and complete healing of this type of wound is of potential clinical significance as the tissues involved are the same as those found in cleft lip. Thus, in families with a high risk of facial clefting, the fetus could be examined directly by fetoscopy. If a cleft were demonstrated, it could be repaired *in utero*. Although this procedure would initially carry a substantially increased risk of morbidity, it would result in the birth of a normally appearing baby. The absence of the deformity might have substantial psychological benefits to the parents and the child.

Acknowledgment: The interest and encouragement by Professor D. E. Poswillo during this study is gratefully acknowledged.

References

ALVARES, O. F., and MYERS, J., Variable features and regional differences in oral epithelium. In: Current Concepts of the Histology of Oral Mucosa. Ed. Squier and Meyer. Springfield, Ill.: C. C. Thomas, 1971.

DIXON, J. B., Inflammation in the fetal and neonatal rat. The local reaction to skin burns, *J. Path. Bact. 80,* 73–82, 1960.

Goss, A. N., Intrauterine healing of fetal rat oral, mucosal, skin, and cartilage wounds, *J. Oral Path. 6,* 35–43, 1977.

HESS, A., Reactions of mammalian fetal tissue to injury. II Skin, *Anat. Rec. 119,* 435, 1954.

LEHMANN, G., and KNOEFFEL, P. K., Trichlorethanol, Tribromethanol, Chloral Hydrate and Bromal Hydrate, *J. Pharmacol. Exp. Ther., 63,* 453–465, 1938.

ROSS, R., Wound Healing, *Sci. Am.*, 40–50, June, 1969.

SOMASUNDARAM, K., and PRATHAP, K., Intrauterine healing of skin wounds in rabbit fetuses, *J. Path. 100,* 81–86, 1970.

SOMASUNDARAM, K., and PRATHAP, K., The effect of exclusion of amniotic fluid on intrauterine healing of skin wounds in rabbit fetuses, *J. Path., 107,* 127–130, 1972.

SOPHER, D., Future prospects for fetal surgery. In: Teratology: Trends and Applications. Burry, C. L. and Poswillo, D. E. (Eds.), Ch. 10. New York: Springer-Verlag, 1975.

TOTO, P. D., and HAWEN, A. D., Generation cycle of Oral Epithelium in 400-day-old mice, *J. Dent. Res., 45,* 948, 1966.

VIZIAM, C. B., MATOLSKY, A. G., and MESCON, H., Epithelialisation of small wounds, *J. Invest. Dermatol. 43,* 499–507, 1964.